multipotency and the molecular distinctions between multipotent versus restricted progenitors are poorly understood. Using genetic and in vitro manipulations we demonstrate here that the stem cell protein Foxd3 is required for self-renewal and maintenance of multipotency in the neural crest. In the overlapping cardiac and vagal neural crest domains, Foxd3 mutant neural crest generated myofibroblast progenitors located ectopically in the distal aorta, and neural derivatives were lost, suggesting alterations in cell fate. Individual neural crest cells were no longer multipotent and did not self-renew normally, failed to maintain stem cell marker expression, and myofibroblast-restricted progenitors were over-represented in mutant cultures. Together, our data show that Foxd3 maintains stem cells of the neural crest by repressing differentiation into non-neural lineages, drawing important parallels between neural crest and other stem cell populations that depend on conserved regulatory mechanisms for control of these defining stem cell characteristics.

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Program/Abstract # 147
Loss of Snail1 leads to loss of mesoderm and neural crest in Xenopus
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In previous studies, we established that blastula/gastrula stage expression of *slug*/(*snail2*) and *twist* are required i) to maintain levels of *slug*/*snail2*, *snail1*, and *twist* RNAs and ii) that injection of *slug*/*snail2* or *twist* RNAs could rescue the Twist and Slug morpholino-induced mesodermal, neural crest, and craniofacial phenotype (see Zhang & Klymkowsky, 2009. Dev. Biol. 331:340). Here we complete this analysis by presenting data that morpholino-based loss of function of *snail1* expression produces a phenotype similar to that seen in *slug*/*snail2* and *twist* morphant embryos, namely loss of mesodermal markers, expansion of endodermal markers, reduction of *snail2*/*slug* and *twist* RNA levels, and loss of neural crest markers. These effects could be partially rescued by the injection of either *twist* or *slug*/*snail2* RNAs. These data support a model in which *slug*/*snail2*, *snail1*, and *twist* expression are dependent upon one another in the early embryo, and required for mesoderm specification. This work is supported by NIH grant 84133.

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Program/Abstract # 148
Loss of early mesoderm leads to loss of neural crest
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Spurred by the report that mesodermal signals are not required for neural crest induction in the zebrafish (Ragland & Raible. 2004 Dev. Biol. 276:16), together with our own observations that loss of blastula/early gastrula-stage expression of *slug*/(*snail2*), *twist*, *or* snail1 led to the loss of both mesoderm and neural crest (Zhang et al., 2006. PLoS ONE, 1:e106; Zhang & Klymkowsky, 2009. Dev. Biol. 331:340; Shi et al., in preparation), we examined how loss of early mesoderm in *Xenopus laevis* influences neural crest formation. Both morpholino-based inhibition of the mesodermally-expressed T-box transcription factors Brachyury (Xbra) and Antipodean/VegT or RNA-based expression of deltaNp63, which antagonizes p53-dependent TGFbeta-signaling (Barton et al., 2009. Dev. Biol. 329:130) led to the loss of mesoderm and neural crest. Xbra/Antipodean appear to be part of the early embryonic Slug/Snail/Twist network, since i) loss of Slug/Snail2, Snail1 or Twist led to reduction in Xbra and Antipodean/VegT RNA levels; ii) loss of Xbra/Antipodean led to a decrease in Slug/Snail2-Snail1-Twist RNA levels, and iii) injection of Slug/Snail2 or Twist RNAs rescued the Xbra/Antipodean morpholino phenotype. These data clearly indicate that, in vivo, neural crest formation is totally dependent upon signals from mesoderm, and that the mesoderm specification network is highly interconnected. This work is supported by NIH grant 84133.

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Program/Abstract # 149
Towards understanding the prdm1a gene regulatory network in *Danio rerio*
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Proper migration of cranial neural crest cells (cncc) into the pharyngeal arches and subsequent interaction with the surrounding environment is necessary for formation of the adult craniofacial skeleton. *prdm1a* is a zinc-finger containing transcription factor that has previously been shown to be expressed in the cncc and posterior pharyngeal arches in *Danio rerio*. Loss of *prdm1a* function, by morpholino injection or in the *narrowminded* mutant, results in a loss of cncc posterior arch derived structures, including ceratobrachials 2–5. To date, little is known about signaling downstream of *prdm1a* in neural crest cells and craniofacial development. Taking a more molecular based approach, we are interested in determining genes that are activated directly downstream of Prdm1a during craniofacial development. We show that Prdm1a protein expression closely resembles the mRNA expression in time-course experiments. Prdm1a protein expression begins at tailbud stage, peaks around 15 somites, begins to decrease and it then peaks again at approximately 31 hpf. These data suggest that there may be early and late roles for *prdm1a* in craniofacial development. Our goal is to use chromatin immunoprecipitation (ChIP) and ChIP-sequencing to elucidate the Prdm1a gene regulatory network.

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Program/Abstract # 150
prdm genes in zebrafish craniofacial development
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The zebrafish *prdm* gene family is comprised of a set of 17 genes that all contain an N-terminal PR/SET domain to mediate protein interactions, and a variable number of DNA-binding zinc finger domains. *prdm1a*, a member of the *prdm* gene family, exhibits craniofacial defects including missing posterior ceratobranchial cartilages. However, the potential roles of other members of this gene family in zebrafish craniofacial development are currently unknown. We hypothesize that multiple *prdm* genes are involved in the patterning and development of the zebrafish face. Gene expression analysis of candidate *prdm* genes identified three novel targets — *prdm3*, *prdm5* and *prdm16*. These genes are expressed in temporal–spatial patterns consistent with putative involvement in craniofacial development. *prdm3* and *prdm16* are expressed in specific neural and branchial arch domains at both 24 and 48 h post fertilization (hpf), while *prdm5* is expressed at low levels ubiqui-